Geoscience Frontiers 4 (2013) 633–646

Contents lists available at SciVerse ScienceDirect

China University of Geosciences (Beijing)

Geoscience Frontiers

journal homepage: www.elsevier.com/locate/gsf

Research paper

Biogeographical note on Antarctic microflorae: Endemism and cosmopolitanism

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ARTICLE INFO

Article history:
Received 22 May 2012
Received in revised form
14 October 2012
Accepted 2 November 2012
Available online 23 November 2012

Keywords:
Biogeography
Cyanobacteria
Halophilic bacteria
16S rRNA
Molecular phylogeny

ABSTRACT

This study deals with the biogeography of Antarctic microflora (Antarctica acts as best model to study microbial biogeography) such as cyanobacteria and selected halophiles with special emphasis on *Halomonas variabilis* and *Bacillus licheniformis*. Halophiles are known to be resistant not only to salt stress, but also to extreme temperature, pressure, and aridity and they are capable of surviving in harsh environments such as polar regions, deep-sea habitats, and deserts. Many microbes are known to be resistant to hostile environmental conditions, and are capable of surviving in harsh environments. Our group has isolated 444 strains belonging to 28 genera of halophiles from various environments around the world. The 16S rRNA gene sequences revealed that many of the isolated strains from geographically distant habitats having different environmental conditions, were closely related to each other, with some strains possessing 100% identical sequences. Organisms possessing survival mechanism such as spore formation are usually ubiquitous. The genus *Halomonas* is represented by potentially endemic strains and the ubiquitous *H. variabilis*, while spore-forming *B. licheniformis* showed cosmopolitan distribution. One potentially endemic (moderate endemicity that is regional and/or continental distribution) strain was reported from Syowa station, East Antarctica, and Mario Zucchelli station, West Antarctica, which are geographically separated by 3000 km. Moreover, 15 strains having 100% similarity with *B. licheniformis* were considered cosmopolitans. The results of this work provide support for the middle-ground model that some microbes have moderate endemicity and others have cosmopolitan distribution. These results will contribute to a greater understanding of microbial biogeography with special emphasis on Antarctica.

1. Introduction

Among all continents, Antarctica has the more recent contact with humans. The first ever landing of humans on the continent is no more than two centuries ago while East Antarctica hosted humans only a century ago. It is characterized by extremely dry cold weather, high UV radiation, gusting winds and the terrain is snow-capped most of the time presenting a hostile environment for living organisms. However, a handful of organisms such as bacteria and algae have dominated the continent followed by lower plants (lichens and mosses) thriving on this continent (Namsaraev et al., 2010).

Microbes are considered to be the most abundant and diverse organisms on Earth (Whitman et al., 1998), but little is known about their distribution pattern and biogeography within and between major habitats. This is because, in the past, little importance has been given to microbial biogeography, which has now turned out to be of great importance due to (1) potentially vast microbio-diversity; (2) protection of potentially endangered bacterial species (Staley, 1997); and (3) ecological roles of yet-to-be-studied species. It is now known from increased research explorations to probe microbial diversity and biogeography (Naganuma and Wilmotte, 2009; Vyverman et al., 2010) that a great number of microorganisms inhabit Antarctica (Namsaraev et al., 2010).

A long-held assertion about microbial ecology is that "*everything is everywhere*, but, *the environment selects*" (*EiE*; Baas-Becking,

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Peer-review under responsibility of China University of Geosciences (Beijing).

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http://dx.doi.org/10.1016/j.gsf.2012.11.002

1934) and this has been postulated to address ubiquity of microbes. The hypothesis was abetted by the statement that microbes being tiny, their abundance on Earth, spore-forming ability and short generation time facilitated their worldwide dispersal. Advocates of the ubiquity axiom believed that all microbes are ubiquitous, as microbes are large in number and are easily disseminated globally (Vanormelingen et al., 2008) by many vectors such as water, wind and animals. The notion *EiE* defies the defined geographic distribution of microbes (Vanormelingen et al., 2008) as it was assumed that ubiquitous distribution is possible in organisms less than 1 mm in size (Fenchel and Finlay, 2004). According to Whitfield (2005), the ubiquity of microbes is summarized in the first part of the Baas-Becking's hypothesis "everything [microbes] is everywhere".

However, the endemicity model is in direct contrast with the ubiquity model suggesting that some free-living microorganisms do possess distinct geographical patterns (Martiny et al., 2006). According to Foissner (2006), it is highly unlikely that all microbes are ubiquitous as a result of our sketchy understanding of microbial diversity. Thus, Baas Becking's dictum is no more than an assumption. In contrast to *EiE*, Papke and Ward (2004) have argued that geographic barriers to microbial dispersal are relatively common and physical isolation is an important driver of microbial evolution. According to the endemicity theory some microbial species have special environmental requirements which are not commonly encountered throughout the globe such as extreme habitats (hot springs, cryosphere, and hyperhalophilic habitats). The potential dispersal of microbial species of these habitats is much lower than those of non-extremophiles. This limited distribution might promote local distribution creating distinct local populations (Whitaker et al., 2003; Vyverman et al., 2007; Rossello-Mora et al., 2008). This model suggests that it might be possible for some microbes to be endemic in some areas.

Recently the debate on microbial biogeography is giving more attention focusing on both tenets. The supporters of endemicity suggest that most bacterial species have restricted geographic ranges (Nemergut et al., 2011), while proponents of the ubiquity hypothesis believe in the ubiquity of microbes, where they propose that there is no biogeography for anything less than 1 mm (Fenchel and Finlay, 2004). These apparently contradictory examples suggest that, possibly, both ideas are true (Ragon et al., 2012). Hence, a new concept of middle-ground model (cosmopolitanism and moderate endemicity) is gaining support with the spectrum of potential microbial biogeography in the middle and with ubiquity and endemicity models at the extreme ends. A conservative middle-ground model (cosmopolitanism) is explained in the second half of Baas-Backing's hypothesis: "everything is everywhere; *the environment selects*" (Whitfield, 2005). This suggests that microbes potentially and in reality have ubiquitous distribution if the ideal environmental conditions are provided. This caveat infers that similar microbial community may occur in similar habitats around the globe, at least below the aforementioned size. The other middle-ground model that is "moderate-endemicity" believes that most but not all of the microbes may be cosmopolitan in distribution due to low transportability, tolerance, and adaptability (Foissner, 2007). This model has unique features and behaves differently from other models of biogeography; it is more flexible and does not lump all microbes under a single model of biogeography. According to "moderate-endemicity", wide dispersal of some microbes is possible as a result of their small size, survivability, ease of transport and early colonization and rapid growth. A study of ultramicrobacteria conducted by Nakai et al. (in press) showed that some of these bacteria are distributed globally, while other show restricted distribution.

The main reasons of discrepancy in microbial biogeography include lack of data, lack of a unifying species concept for prokaryotes and the absence of universally accepted methods for microbial biogeographic studies. As a result, our information on the biodiversity and distribution of Antarctic microflorae is so far mainly limited to traditional microbial identification based on morphological characteristics. To solve these problems, molecular techniques were applied which have boosted our understanding of microbial diversity, which in turn helped to describe their biogeography. For example, the 16S rRNA gene, which has become one of the primary tools in bacterial classification, determines the genetic diversity of complex bacterial populations based on comparison of level of similarity between bacterial DNA sequences. However, these census techniques have their own limitations and could not develop discrete consensus on microbial biogeography so far. As an example, one of the limitations of the 16S rRNA is providing only partial information of the prokaryotic genome, instead of giving full-length genome amplification (Shah et al., 2011). Similarly, the presence of multiple copies of 16S rRNA in bacterium (Dahllöf et al., 2000) and chimeric sequences (Shah et al., 2011) severely hampers community analysis and may lead to biased results. Although there is increased precision of molecular methods, there is no comprehensive single methodology which can reveal the presence of every single organism in a given environmental sample. One of the serious problems in addressing microbial biogeography using available molecular techniques is their inability to detect rare taxa in the environment. The number and variety of less abundant taxa present in any given environmental sample is large (Ashby et al., 2007). This limitation of current microbial methods presents hurdles in the detection of rare taxa and in understanding microbial biogeography on a global scale. So these methods should be used with great caution while comparing communities between environmental samples, especially when species of interest are rare in any of these samples.

Besides their weakness, the application of molecular methods resulted in the increase of polar microfloral biodiversity database and suggesting a degree of endemism (Taton et al., 2006; Laybourn-Parry and Pearce, 2007; Hobbie and Laybourn-Parry, 2008; Vyverman et al., 2010; Fernandez et al., 2011; Margesin and Miteva, 2011), which may be due to isolation and the unique habitats in these regions. The geographic isolation and evolutionary history of Antarctica produced a unique habitat supporting endemicity of microbes (Fernandez et al., 2011). However, there is need to be careful as global microbial data still have to be unveiled and available gene databank is incomplete. For example three taxa previously considered as endemic to Antarctica showed more than 99% similar to sequences found in High Arctic Canada (Jungblut et al., 2010). It is noteworthy that some microbes (particularly aerially dispersed) which can go dormant for extended periods of time and survive harsh environmental conditions are more likely to be transported for long distances (Jones and Harrison, 2004). The smooth but rapid (up to 240 km/h) wind flow of the troposphere carrying dust particles and microbes can facilitate dissemination of microbes across the continents in as little as a few days. Particles including microbes at this altitude may be carried from adjacent land masses such as Patagonia and the Falkland Islands to the Antarctic coast and interior (David and Williams, 1991). It is not difficult for aerially transported microbes of the mid latitudes to travel to Antarctica. On landing in Antarctica, viable microbes which are more tolerant to harsh environments might colonize successfully at the onset of favorable conditions. The inter-continental movement of microbes suggested that there is nothing far for microbes. So the isolation and remoteness only do not support the idea of endemicity in microbes, and that they must be accompanied by some other forces shaping the potential endemicity of microbes in Antarctica.

On the other hand some genera like *Halomonas* of the *gammaproteobacteria* group host representatives of both potentially endemic as well as cosmopolitan strains isolated from Antarctica. These findings support the concept of the middle-ground model, which states that some microbes may have moderate endemicity while others are cosmopolitans. It is widely accepted that *Halomonas variabilis* has a cosmopolitan distribution (Kaye et al., 2011) and can be isolated from many habitats such as deep-sea vents (Kaye and Baross, 2000; Kaye et al., 2011), hot petroleum reservoirs (Orphan et al., 2000), the Great Salt Lake (Fendrich, 1988), Antarctic sea ice (Bowman et al., 1997) and terrestrial environments in Antarctica (Okamoto et al., 2004). Some strains belonging to genus *Halomonas* have been isolated from saline ponds near Syowa Station, East Antarctica, and near the Mario Zucchelli Station, West Antarctica (Naganuma et al., 2005) and never reported from other parts of the globe so far. There existed no closely related type strains to these newly reported strains anywhere, and homology with most closely related sequence according to the DDBJ/EMBL/GeneBank DNA database was as high as 97.7%. Until now, neither closely related strains nor sequence have been obtained elsewhere, suggesting that these strains may be endemic in the saline ponds of Antarctica.

A spore-forming bacterium *Bacillus licheniformis*, characterized by its tolerance for salinity, high temperature and pressure stress, is of particular interest. Based on these abilities it is thought that *B. licheniformis* has a global distribution and is considered as cosmopolitan. In this study, closely related strains and some having 100% resemblance were isolated from a wide range of habitats throughout the globe such as the Gobi Desert in China, yellow dust blown from China to Japan (Hua et al., 2007), Qaanaaq glacial moraines on Greenland (Yukimura et al., 2009), and Livingstone Island, Antarctica (this study). It is noteworthy that these strains grouped with *B. licheniformis* and are considered cosmopolitan strains. The spore-forming ability of *B. licheniformis* is one of its successful strategies to survive the harsh environments such as drought, extreme temperature, ultraviolet radiations, and extreme oligotrophic conditions (Onyenwoke et al., 2004; Amato et al., 2007; Yukimura et al., 2009). Microbes characterized by spore formation are more likely to be transported long distances and may have wide distribution and considered as cosmopolitan. The wide global distribution of these isolated strains and *B. licheniformis* supports the Baas Becking's hypothesis that everything (some microbes) is everywhere if provided with suitable environmental conditions. In this case, spore formation may help these bacteria to tolerate harsh environmental conditions and which might cause them to germinate on arrival of suitable conditions.

Yet despite much recent information, knowledge about the biogeography of the Antarctic microflora is still in its infancy. The problem lies in the lack of comprehensive literature review on biogeography of microbes; the relevant data are often vague, scattered, and highly prone to error due to flaws in identification methodology (Foissner, 2006). The biogeographical investigation of Antarctic microbial flora is of immense importance for the effective management and conservation of important organisms in the future. Endemic species having small population and narrow distribution and which are inherently more vulnerable to extinction (Gaston, 1998) are important targets for conservation (Myers et al., 2000). Microorganisms have been largely ignored by conservation efforts. Yet their role in biogeochemical processes, their diversity and abundance, and their potential as repositories of valuable genetic information and metabolic products make them as important as animals and plants. However, the arguments for their conservation require careful examination and are not merely an extrapolation of the arguments for animal and plant conservation (Cockell et al., 2009). Antarctic organisms may possess cold tolerant enzymes and bioactive compounds of significant nature, which may have industrial and biotechnological applications. They are not merely useful as our survival depends on them. Hence the conservation of microbes on account of their inherent value is not a controversial motive (Cockell et al., 2009). This article deals with the biogeography of microbes in Antarctica based on available literature and original results (for studied halophiles) collected by our research group from various parts of the world including Antarctica.

1.1. Why "everything is everywhere" is considered as metaphor?

Although much valuable work has been done for the past decade, available data do not clearly support or reject the *EiE* hypothesis. A seminal notion based on the ubiquity dictum, that all microbes are ubiquitously dispersed globally, was challenged by the advent of many molecular techniques and their application in the field of microbial ecology. Now the question is why *EiE* has been criticized? To answer this question, let us discuss some studies supporting the metaphor of *EiE*. For example, Fenchel et al. (1997) conducted their experiment for local *vs*. global diversity measurement of ciliates and found high local/global diversity ratio for ciliates with about 10% of the global ciliate species present in one small freshwater pond. From this study they concluded that microorganisms are present almost everywhere. Later on these results were criticized by Foissner (2006), arguing that the diversity of ciliates is an unknown quantity. According to Foissner et al. (2002) global estimate of ciliate species is much higher (30,000 species) than Finlay's (2001) estimates of 3000 ciliate species globally. According to Foissner flawed results based on Fenchel and Finlay's estimates of ciliate species led to a high local/global diversity ratio. As long as the global diversity of studied organism is unknown, the use of local/global diversity measure is futile (Foissner, 2006). Another example is under-sampling of microbes due to methodological errors and misidentification which leads to the disregard for rare species with distribution data becoming skewed to the ubiquitous and easy to identify species (Foissner, 2006; Petz et al., 2007).

There are microorganisms known to have wide range of distribution throughout the globe. Their ubiquitous distribution supports the first part of the *EiE* hypothesis when provided with the conditions described in the second half of this hypothesis. For example, Arctic and Antarctic regions characterized by similar ecological conditions but separated geographically by climatic barriers (Staley and Gosinks, 1999) were found to harbor representatives of similar genera, representing those members likely to possess similar survival mechanisms (Christner et al., 2008). Petz et al. (2007) identified 334 species of freshwater ciliates in both polar regions and observed 44 species common to both poles. Similarly Jungblut et al. (2010) found a global distribution of cold-adopted cyanobacteria throughout the cold terrestrial biosphere. Besides this, some microbes endemic to Antarctica were found later on to have the highest match (more than 99.6%) to some of the sequences reported from the high arctic regions (Jungblut et al., 2010). As with the development of genetic database the proportion of potential endemism in microbes is decreasing (Fernandez et al., 2011). Apart from these situations, the lack of one standard technique to study microbial biogeography is one of the big hurdles blocking consensus in microbial biogeography. In addition, different techniques may provide different results for the same dataset. For example, Cho and Tiedje (2000) found fluorescent *Pseudomonas* strains with a cosmopolitan distribution based on the results of 16S rRNA gene sequences but genomic fingerprinting revealed a high level of endemicity. Hence, current science is not in a position to completely accept or reject the hypothesis *EiE*. We

need to develop a robust single standard technique to explore the biogeography of microbes.

1.2. Endemism in Antarctica

About two decades ago, the microbial community structure in Antarctica was a mystery with knowledge of microbial distribution based solely on traditional morphological studies. However, due to morphological similarities, it is sometimes difficult to distinguish between two species of microorganisms. To overcome the problem, molecular techniques were used in microbial ecology (Laybourn-Parry and Pearce, 2007). With the sophistication of these techniques, the role of microbes and their biogeography are becoming more clear (Moosvi et al., 2005). As it is a long-held debate whether microbes have restricted geographical distribution or endemic to specific areas or not, many studies tried to answer the question without conclusive results. To find the answers of microbial endemism, Antarctica represents the best study model as it is isolated from rest of the world (Vincent, 2000b).

Antarctica is assumed to be isolated from other landmass as a result of the wide oceanic expansion and has been so for 25–30 million years (Clarke, 2003). It is several hundred kilometers from its closest neighbor. This spatio-temporal isolation of Antarctica from the other continents might have favored the evolution of endemic taxa (Vincent, 2000b; Fernandez et al., 2011) and might have limited the colonization of temporal latitude species that may have given rise to indigenous species (Margesin and Miteva, 2011). It is evident that the local scale dispersal of microbes is more efficient than large-scale dispersal (Vincent, 2000b) because active dispersal is critically constrained in the latter (Jenkins et al., 2007). The isolation of Antarctica from the rest of the world for millions of years and the more efficient dispersal of local species compared to long-distance dispersal may support the concept of potential endemism (Taton et al., 2006). Dispersal itself will not alter biogeographical patterns unless it is accompanied by successful colonization of the new environment. If colonization rates are very low, we would expect to observe endemicity at the community-level (Papke and Ward, 2004).

Antarctica is considered one of the most hostile environments on Earth characterized by extreme cold, dryness, gusting winds, UV radiations and light extremes (i.e., total darkness in winter and permanent light in summer) (Severin and Stal, 2010). This harsh environment is hostile to life in the region; hence only quite a few organisms can survive under such circumstances, resulting in a truncated food web (Laybourn-Parry, 2002). In many lakes, comparatively low temperature, low photosynthetically active radiation (PAR) and meager quantity of nutrients make these habitats severely hostile for organisms. The organisms surviving under such a harsh environment underwent high-pressure selection and developed special survival mechanisms (Van Trappen et al., 2002). For example, phytoplankton residing in poorly illuminated lakes have evolved photo-systems adapted to shade and feeding mechanisms known as mixotrophy (Marshall and Laybourn-Parry, 2002). These organisms have evolved morphologically and functionally to sustain their life in such a hostile environment (Laybourn-Parry and Pearce, 2007; Margesin and Miteva, 2011). These extraordinary features in psychrophiles may have evolved due to strong environmental selection (Vincent, 2000b; Poli et al., 2007), rendering these organisms genetically divergent from their close non-polar relatives.

Based on these findings it may be assumed that there would have been special microbial masses that evolved in these extremely cold habitats and may represent potential endemism (Laybourn-Parry and Pearce, 2007; Fernandez et al., 2011). An investigation carried out by Petz et al. (2007) identified 334 species of freshwater ciliates in both polar regions and observed 44 species common to both poles. Over 20% of the taxa were found to be new to science in any one of the investigated areas. The differences in species composition between both polar regions suggested a restricted distribution of freshwater ciliates instead of an ubiquitous nature.

Microbial endemism is argued based on the isolation and extreme environmental conditions of Antarctica. However, the point here is to identify what is far for a microbe? Microbes, due to their high abundance and small sizes, have the ability to disperse with great frequency from one part of the world to another over long distances through passive means (i.e., winds, water currents and humans) (Vanormelingen et al., 2008; Watts et al., 2011; Wilkinson et al., 2012). Although, differences among different groups of microbes may affect their relative transportability in a measurable way (Litchman, 2010), it is possible for some microbes from the low latitudes to reach geographically isolated Antarctica. A study conducted by Whitaker (2006) suggested that spore-forming and motile organisms are able to disperse freely and would be more likely to be cosmopolitan. Taton et al. (2006) found operational

Table 1
List of bacteria present in different habitats of Antarctica as determined by the rRNA approach.

	Soil	Continental shelf sediment		Cyanobacterial mat sample	Subglacial ice	Lake sediment	Saline lake sediment
α-Proteobacteria	1	1	1	1	1	0	1
β-Proteobacteria	1	1	1	1	1	1	1
γ-Proteobacteria	1	1	1	1	0	0	1
δ-Proteobacteria	0	1	1	1	0	0	1
ε-Proteobacteria	0	1	1	0	0	0	0
Acidobacteria	1	0	1	0	0	1	0
Gemmatimonadetes	1	0	0	0	0	0	0
Bacteroidetes	1	0	0	1	0	0	0
Actinobacteria	1	1	1	1	1	1	1
Chloroflexi	1	0	0	0	0	0	0
Chlamydiae	1	1	1	1	0	1	1
Nitrospira	1	0	0	0	0	0	0
Clostridium-Bacillus	0	0	0	1	0	0	1
Cytophaga-Flavobacteria	0	1	1	1	0	0	1
Spirochaetales	0	0	1	0	0	0	1
Green non-sulphur	0	1	1	0	0	1	0
Planctomycetales	0	1	1	0	0	1	0
Cyanobacteria	0	0	0	0	0	1	1
OP11Group/others	0	0	1	1	0	1	0
Uncultured	1	0	0	0	0	0	1
Archaea	NA	1	1	1	NA	NA	1

The original table that showed the percentage of these phyla in different habitat was modified in current paper and we just focused on the presence absence data.

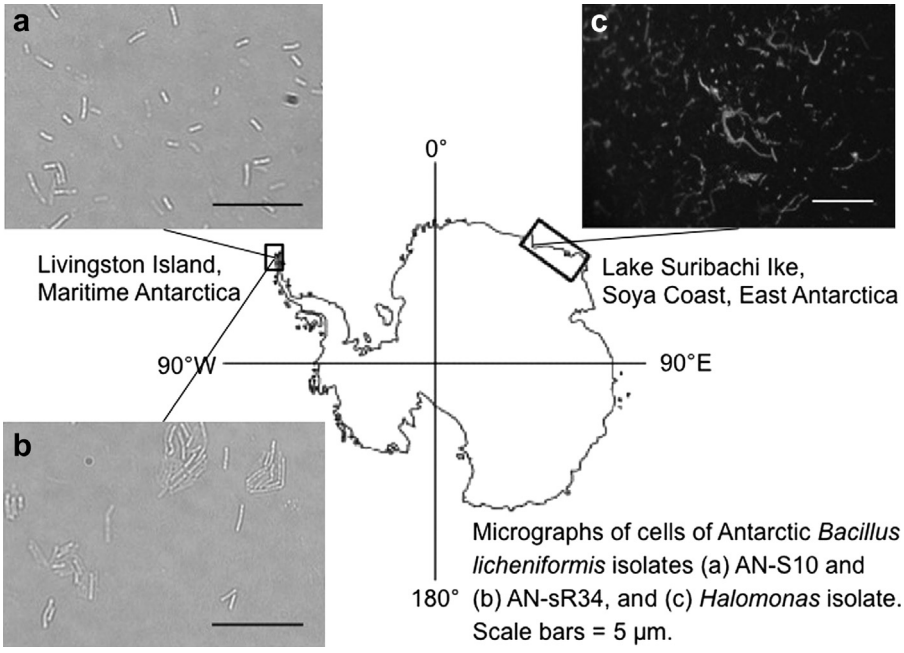


Figure 1. Location map of studied sites in Antarctica.

taxonomic units which are also found outside Antarctica that were more widespread over the frozen continent than potential endemics. This situation indicates that these OTUs may be well adapted to transport and dispersal and thus were quite successful in spreading and colonizing new Antarctic habitats or vice versa. Hence, endemism of microbes in Antarctica should be considered with great caution, as microbes can travel long distances and have strategies to tolerate harsh environments. As genetic databases grow, they may introduce gene sequences from other parts of the world which might have similarity to species previously considered as endemic to Antarctica.

1.2.1. Endemism in Antarctic cyanobacteria

In the Antarctic region many researchers have focused on the cyanobacteria to explore and expand the knowledge base of bacterium in the region (Vincent, 2000a,b; Taton et al., 2003, 2006; Vyverman et al., 2010; Fernandez et al., 2011). Cyanobacteria was found to be the most successful autotrophic group in the region

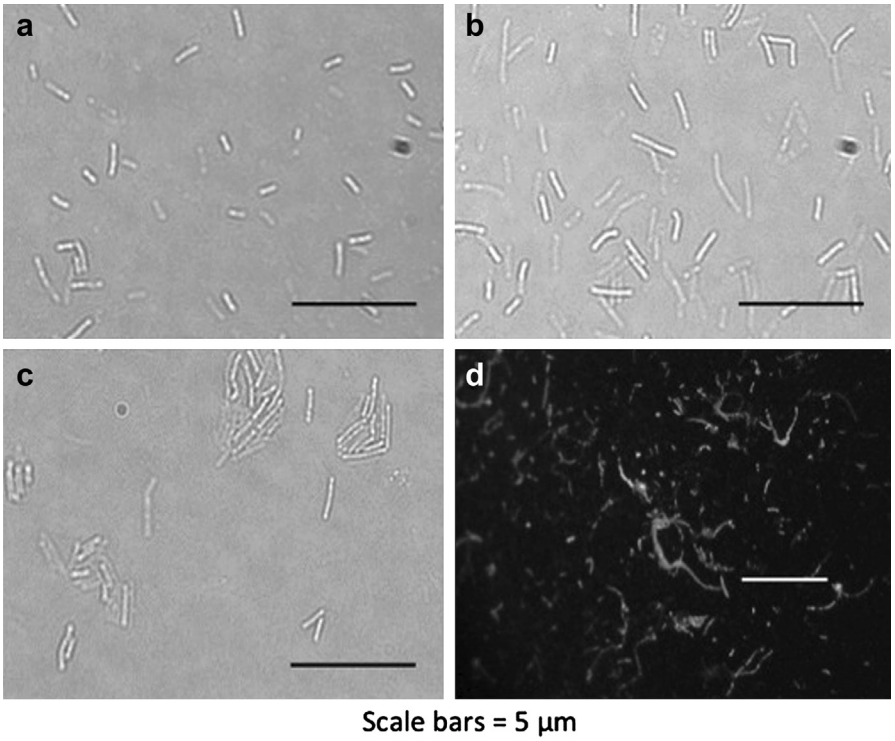
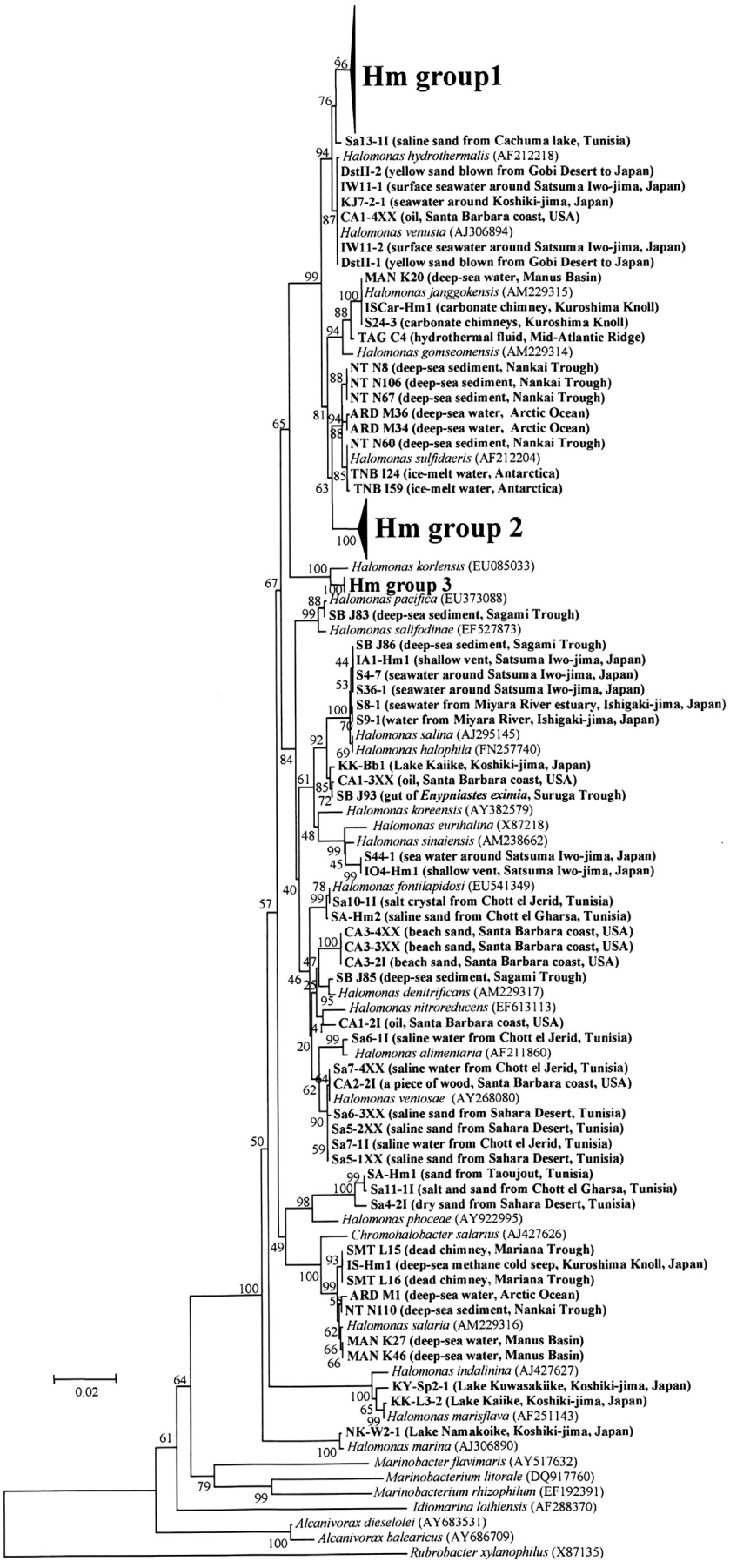


Figure 2. Micrographs of cells of Antarctic *Bacillus licheniformis* isolates (a) AN-S10, (b) AN-sR16, and (c) AN-sR34; and (d) *Halomonas* isolate.


Hm group1
Sa13-1I (saline sand from Cachuma lake, Tunisia)
Halomonas hydrothermalis (AF212218)
DstII-2 (yellow sand blown from Gobi Desert to Japan)
IW11-1 (surface seawater around Satsuma Iwo-jima, Japan)
KJ7-2-1 (seawater around Koshiki-jima, Japan)
CA1-4XX (oil, Santa Barbara coast, USA)
Halomonas venusta (AJ306894)
IW11-2 (surface seawater around Satsuma Iwo-jima, Japan)
DstII-1 (yellow sand blown from Gobi Desert to Japan)
MAN K20 (deep-sea water, Manus Basin)
Halomonas janggokensis (AM229315)
ISCar-Hm1 (carbonate chimney, Kuroshima Knoll)
S24-3 (carbonate chimneys, Kuroshima Knoll)
TAG C4 (hydrothermal fluid, Mid-Atlantic Ridge)
Halomonas gomseomensis (AM229314)
NT N8 (deep-sea sediment, Nankai Trough)
NT N106 (deep-sea sediment, Nankai Trough)
NT N67 (deep-sea sediment, Nankai Trough)
ARD M36 (deep-sea water, Arctic Ocean)
ARD M34 (deep-sea water, Arctic Ocean)
NT N60 (deep-sea sediment, Nankai Trough)
Halomonas sulfidaeris (AF212204)
TNB I24 (ice-melt water, Antarctica)
TNB I59 (ice-melt water, Antarctica)
Hm group 2
Halomonas korlensis (EU085033)
Hm group 3
Halomonas pacifica (EU373088)
SB J83 (deep-sea sediment, Sagami Trough)
Halomonas salifodinae (EF527873)
SB J86 (deep-sea sediment, Sagami Trough)
IA1-Hm1 (shallow vent, Satsuma Iwo-jima, Japan)
S4-7 (seawater around Satsuma Iwo-jima, Japan)
S36-1 (seawater around Satsuma Iwo-jima, Japan)
S8-1 (seawater from Miyara River estuary, Ishigaki-jima, Japan)
S9-1(water from Miyara River, Ishigaki-jima, Japan)
Halomonas salina (AJ295145)
Halomonas halophila (FN257740)
KK-Bb1 (Lake Kaiike, Koshiki-jima, Japan)
CA1-3XX (oil, Santa Barbara coast, USA)
SB J93 (gut of Enypniastes eximia, Suruga Trough)
Halomonas koreensis (AY382579)
Halomonas eurihalina (X87218)
Halomonas sinaiensis (AM238662)
S44-1 (sea water around Satsuma Iwo-jima, Japan)
IO4-Hm1 (shallow vent, Satsuma Iwo-jima, Japan)
Halomonas fontilapidosi (EU541349)
Sa10-1I (salt crystal from Chott el Jerid, Tunisia)
SA-Hm2 (saline sand from Chott el Gharsa, Tunisia)
CA3-4XX (beach sand, Santa Barbara coast, USA)
CA3-3XX (beach sand, Santa Barbara coast, USA)
CA3-2I (beach sand, Santa Barbara coast, USA)
SB J85 (deep-sea sediment, Sagami Trough)
Halomonas denitrificans (AM229317)
Halomonas nitroreducens (EF613113)
CA1-2I (oil, Santa Barbara coast, USA)
Sa6-1I (saline water from Chott el Jerid, Tunisia)
Halomonas alimentaria (AF211860)
Sa7-4XX (saline water from Chott el Jerid, Tunisia)
CA2-2I (a piece of wood, Santa Barbara coast, USA)
Halomonas ventosae (AY268080)
Sa6-3XX (saline sand from Sahara Desert, Tunisia)
Sa5-2XX (saline sand from Sahara Desert, Tunisia)
Sa7-1I (saline water from Chott el Jerid, Tunisia)
Sa5-1XX (saline sand from Sahara Desert, Tunisia)
SA-Hm1 (sand from Taoujout, Tunisia)
Sa11-1I (salt and sand from Chott el Gharsa, Tunisia)
Sa4-2I (dry sand from Sahara Desert, Tunisia)
Halomonas phoceae (AY922995)
Chromohalobacter salarius (AJ427626)
SMT L15 (dead chimney, Mariana Trough)
IS-Hm1 (deep-sea methane cold seep, Kuroshima Knoll, Japan)
SMT L16 (dead chimney, Mariana Trough)
ARD M1 (deep-sea water, Arctic Ocean)
NT N110 (deep-sea sediment, Nankai Trough)
Halomonas salaria (AM229316)
MAN K27 (deep-sea water, Manus Basin)
MAN K46 (deep-sea water, Manus Basin)
Halomonas indalinina (AJ427627)
KY-Sp2-1 (Lake Kuwasakiike, Koshiki-jima, Japan)
KK-L3-2 (Lake Kaiike, Koshiki-jima, Japan)
Halomonas marisflava (AF251143)
NK-W2-1 (Lake Namakoike, Koshiki-jima, Japan)
Halomonas marina (AJ306890)
Marinobacter flavimaris (AY517632)
Marinobacterium litorale (DQ917760)
Marinobacterium rhizophilum (EF192391)
Idiomarina loihiensis (AF288370)
Alcanivorax dieselolei (AY683531)
Alcanivorax balearicus (AY686709)
Rubrobacter xylanophilus (X87135)
0.02

(Jungblut et al., 2005; Taton et al., 2006). In Antarctica, cyanobacteria have been isolated from the bottoms of inland waters (i.e., streams, lakes and pools) where they occur as microbial mats; from water columns, soils, cryoconite holes, cryptoendolithic habitats and rock surfaces (Vincent, 2000a; Bargagli, 2005; Severin and Stal, 2010). However, the biogeography of different taxa of cyanobacteria is still in its infancy and provides the grounds for heated debates. The initial attempts to study the biogeography of cyanobacteria in Antarctica were totally based on morphological characteristics (Vincent, 2000a), and owing to the morphological plasticity of cyanobacteria has led to vague results: that there is a rare chance of endemism in polar cyanobacteria (Zakhia et al., 2008). Besides this, Komárek (1999) claimed that Antarctic taxa have been identified using taxonomic keys developed for temperate and tropical microflora, which assigned cosmopolitan distribution of these taxa. However, the combination of phenotypic and genotypic approaches to study cyanobacteria resulted in a modern classification system (Komárek et al., 2008) that changed the previous views of no endemism in Antarctic cyanobacteria.

The first ever culture-independent molecular ecological investigation was conducted to study the phenotypic and genotypic biodiversity of cyanobacteria in field microbial mat samples of Lake Fryxell and in artificial culture using the benthic gradient chamber (BGC) (Taton et al., 2003). This study showed a total of eight morphotypes with one endemic morphotype to Antarctica based on the phenotypic approach, while molecularly aided approach yield entirely different results with higher number (15) of phylotypes. The results of 16S rRNA gene sequence showed three Antarctic lineages and two novel lineages out of a total 11 lineages transcribed. It was suggested that endemism in Antarctic cyanobacteria could be higher than what has been estimated on the basis of morphological features alone (Taton et al., 2003). Similar results were obtained by Jungblut et al. (2005) and Taton et al. (2006) who suggested high rates (57.1%) of potential Antarctic endemic cyanobacterial species. Gibson et al. (2006) assessed the distribution of Antarctic cyanobacteria by comparing web-based database of rRNA gene sequences with sequence data of cultured and uncultured strains of microbial mats from Antarctica reported by many previous studies (e.g., Vincent, 2000a,b; Nadeau et al., 2001; Taton et al., 2003). They identified a total of 53 operational taxonomic units (OTUs), of which 38 OTUs were totally new and have never been recorded outside Antarctica. Similarly, some recent studies (Vyverman et al., 2010; Fernandez et al., 2011; Margesin and Miteva, 2011) showed biogeographical patterns (e.g., potential endemism) in cyanobacteria that could be the result of geographical isolation (Vyverman et al., 2010).

However, three taxa of cyanobacteria previously identified as endemic to Antarctica (Komárek, 1999; Taton et al., 2006) were found to have more than 99% similar sequences from the Arctic region of Canada (Jungblut et al., 2010). Cyanobacteria isolated from cold habitats have optimum growth rate at 15–20 °C, suggesting that they might have their evolutionary origins in temperate areas that later colonized these cold habitats (Jungblut et al., 2010). A recent study documented early Eocene climate on Antarctica and found that it was characterized by 16 °C mean annual temperature (MAT) and mesothermal to megathermal taxa of modern subtropical to tropical settings in Australia, New Guinea and New Caledonia (Pross et al., 2012). It is then possible that during the Eocene era cyanobacteria present on Antarctica were similar to those found in low latitudes today, as MAT of 16 °C which could have encouraged the growth of those cyanobacteria. The potential endemism is decreasing in Antarctica, as Taton et al. (2006) suggested that 57% potential endemic OTUs of cyanobacteria has been reduced to 28.5% due to increasing database since then (Fernandez et al., 2011). The growing database has recorded similar sequences of many taxa from other parts of the world which were previously recognized as endemic to Antarctica. Similarly, 11 out of total 12 OTUs in Antarctica were characterized as cosmopolitan in distribution and only one OTU was found to be potentially endemic to Antarctica (Fernandez et al., 2011). Although according to Fernandez et al. (2011) one OTU was potentially endemic, however much caution was taken place to describe this OTU endemic to Antarctica, as it may be present in any other part of the world. Hence it is more appropriate to use the word “potentially endemic” rather than endemic.

Besides cyanobacteria there are many members of other microbial flora present in Antarctica. One significant study shedding light on Antarctic microbial diversity listed eight bacterial phyla (viz., *Protobacteria*, *Acidobacteria*, *Bacteroides*, *Actinobacteria*, *Chloroflexi*, *Chlamydiae*, *Nitrospira* and *Gemmatimonadetes*) other than cyanobacteria in soil from the oasis region (Shivaji et al., 2004). The list of genera reported by Shivaji et al. (2004) and some other studies is shown in Table 1. Data show the presence of these bacterial phyla in different habitats of Antarctica, but silent regarding the biogeography of these phyla in this region. About two decades before, there were few studies conducted on microbial inventory and biogeography, however, with the passage of time the microbial database from Antarctica has increased considerably. The data had some shortcomings as the focus is still on the cyanobacteria and the other drawback is that data is scattered and in its infancy. Hence, it is difficult to make concrete statements about the diversity and biogeography of microbes present in Antarctica.

2. Materials and methods

2.1. Sample collection and isolation

Our research group collected halophilic bacteria from a wide range of habitats, including soil and water from deep-seas, deserts and polar regions (Table S1). Our study sites in Antarctica are Livingston Island, Maritime Antarctica and Lake Suribachi Ike, Soya Coast, East Antarctica (Fig. 1). The isolated strains from these two studied sites of Antarctica were *B. licheniformis* and *Halomonas*. The environmental parameters were broad: geographically, isolates were collected from between latitudes 79°N and 74°S; at water depth of 0–7781 m; temperature of sub-zero to over 100 °C; from 0% salinity to saturation; and from oxidative to strongly reductive conditions.

Collected samples were brought to the laboratory for further analyses. Halophiles were isolated from collected raw environmental samples following Okamoto et al. (2004). For the collection of high heat tolerant spore-forming halophiles, the medium was heated at 80 °C for at least 10 min, which gave selective screening (Krieg, 1981). After 2 weeks these enrichment cultures were spread on 1.5% agar plates. This process was repeated three times to get pure colonies.

2.2. Phylogeny

Genomic DNA was extracted according to the procedure of Okamoto et al. (2004) followed by amplification of near full-length fragments of the 16S rRNA gene (DeLong, 1992). After specific amplification, PCR products were purified using QIAquick PCR

Figure 3. Neighbor-joining phylogenetic tree showing diversity of isolates affiliated to genus *Halomonas* based on near full-length 16S rRNA gene sequences. The percentages of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. There were a total of 1286 positions in the final dataset. Names and origins of isolates are in bold. The scale bar indicates 0.02 substitutions per nucleotide site.

purification kit (QIAGEN, Maryland, USA). All purified PCR products were sequenced using ABI 3730XL automatic DNA sequencer (Applied Biosystems, Foster, USA). Sequences were obtained by performing BLASTN homology and compared with those from the DDBJ/EMBL/GenBank database. The highly homologous sequences of the type strains and culture strains were aligned using the ClustalX multiple alignment program (Larkin et al., 2007). These sequences were used to construct phylogenetic tree by the neighbor-joining (NJ) algorithms (Saitou and Nei, 1987) using MEGA ver. 4.0 (Tamura et al., 2007). Out of total 444 isolated strains belonging to 28 genera, we will present only two genera (1) *Halomonas* as an example of "endemism to cosmopolitanism" and (2) *Bacillus* as cosmopolitan from Antarctica (Fig. 2).

3. Results and discussion

3.1. Endemism to cosmopolitanism

There are some genera which comprise species of cosmopolitan distribution and at the same time hosting some other species that

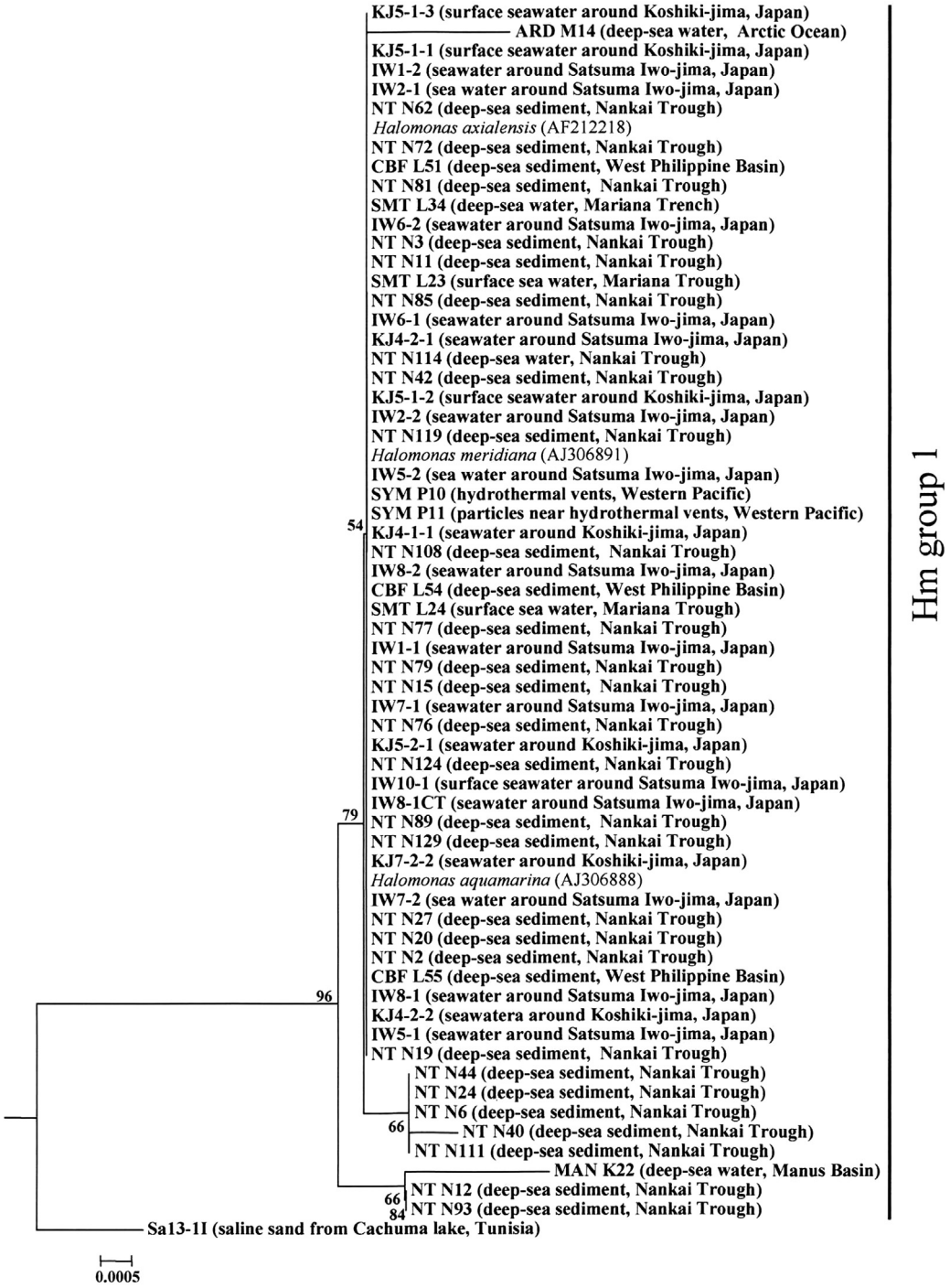


Figure 4. Neighbor-joining phylogenetic tree showing diversity of isolates affiliated to group 1 of *Halomonas* species based on near full-length 16S rRNA gene sequences. The percentages of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. There were a total of 1286 positions in the final dataset. Names and origins of isolates are in bold. The scale bar indicates 0.0005 substitutions per nucleotide site.

are thought to be potentially endemic to particular areas. Here we will discuss the *Halomonas* genus of halophilic bacteria isolated by our group from different habitats including Antarctica.

3.1.1. Isolated strains of the non-spore-forming genus Halomonas

Of the 28 genera of halophiles, most of the isolated strains (152) were representatives of the genus *Halomonas* of the class *Gammaproteobacteria*. Genus *Halomonas* is a typical euryhaline halophile that utilizes ectoine, glycine, betaine, and other substances as osmoregulators (Galinski, 1995; Cánovas et al., 1998) and occurring in various environments. We isolated *Halomonas* in abundance from many habitats including seawater, marine sediments, salt lakes, deserts and from Antarctica.

In this study, the isolated strains of *Halomonas* from various environments were divided into three main groups such as Hm groups 1, 2 and 3, based on their closest known species (Fig. 3). About 60 isolated strains grouped together in Hm group 1, and were closely related to *Halomonas meridiana*, *Halomonas aquamarina*, and *Halomonas axialensis*; and these strains did not constitute any genetically mutated group (Fig. 4). The strains closely related to

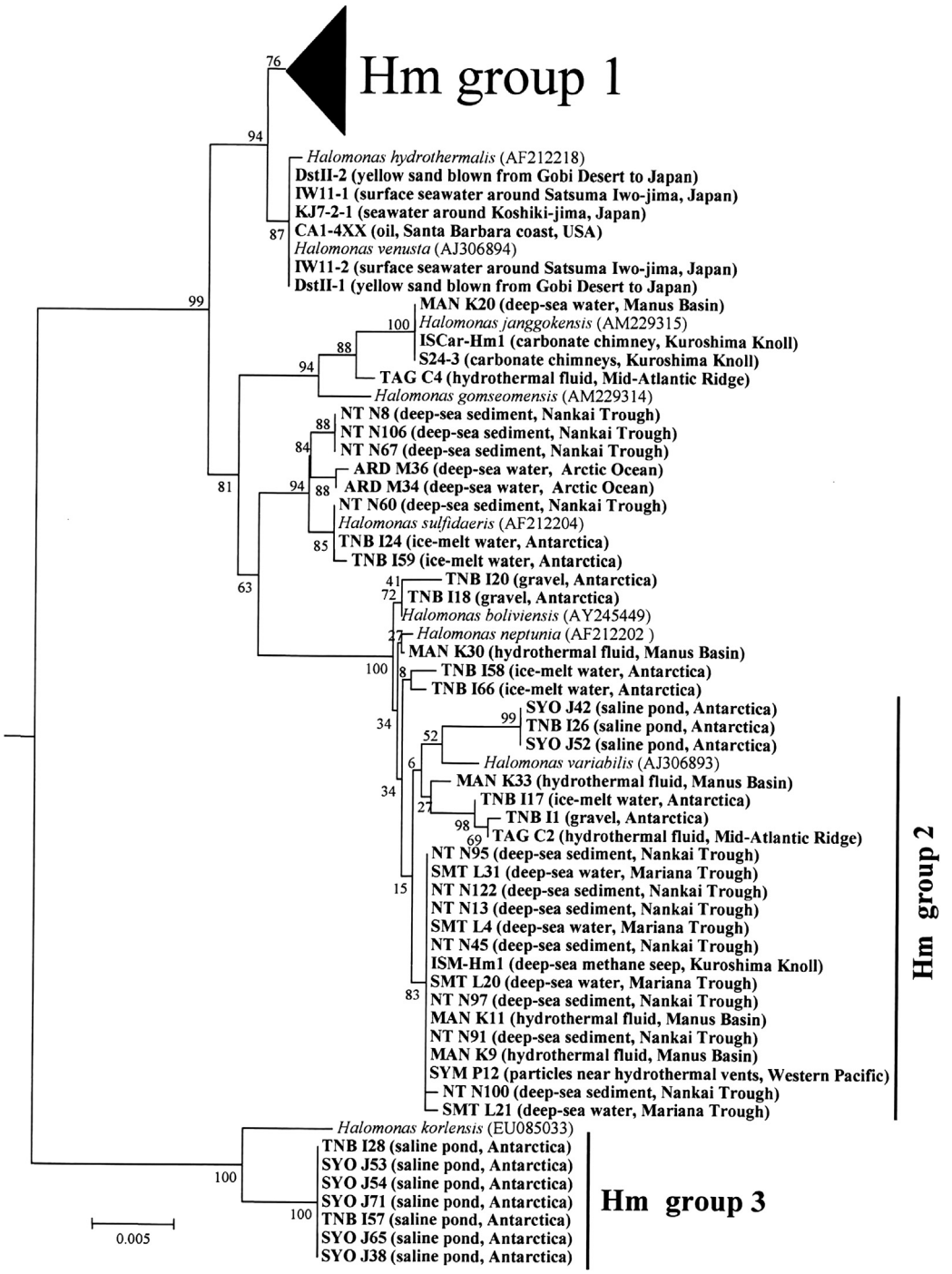


Figure 5. Neighbor-joining phylogenetic tree showing diversity of isolates affiliated to group 2 and group 3 of *Halomonas* species based on near full-length 16S rRNA gene sequences. The percentages of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. There were a total of 1286 positions in the final dataset. Names and origins of isolates are in bold. The scale bar indicates 0.005 substitutions per nucleotide site.

these three species were obtained from a variety of marine environments, such as surface seawater and deep-sea sediments, with the homology shown to be at least 99% between each of the strains. However, we did not conduct physiological experimentation in this study; the precise species to which the isolated strains belong could not be elucidated. Although the geographical distances between the sampling locations of these strains were large, all sources were seawater or deep-sea sediments. In addition to those isolated by our group, strains closely related to these three species have been reported from various oceanic environments (James et al., 1994; Sass et al., 2001), possibly suggesting their cosmopolitan distribution in marine environments.

For strains closely related to *H. variabilis* in the Hm group 2, the homology was as high as 99.93% between strains isolated from deep-sea hydrothermal fluids (TAG C2) and Antarctic strains (TNB I1), which is almost identical in terms of phylogenetics (Fig. 5). The growth patterns of obtained strains from both regions were observed ardently, and were found different in comparison to each other, which ruled out the possibility of cross-contamination in the laboratory (Okamoto et al., 2001). The stress tolerance ability of *H. variabilis* and other *Halomonas* species may facilitate their wide distribution across the globe in a wide range of habitats including the so-called extreme habitats (Okamoto et al., 2004). *H. variabilis* occurs in wide array of environments and was first reported from the Great Salt Lake (Fendrich, 1988), Antarctica (Okamoto et al., 2001, 2004), sea ice (Bowman et al., 1997), both polar seas (Mergaert et al., 2001), deep-sea hydrothermal plumes (Kaye and Baross, 2000; Okamoto et al., 2001, 2004; Kaye et al., 2011) and hot petroleum reservoirs (Orphan et al., 2000), suggesting that *H. variabilis* has a cosmopolitan distribution.

In contrast to the cosmopolitan distribution of the strains belonging to Hm groups 1 and 2, Hm group 3 consisted solely of potentially endemic strains obtained from saline ponds near the Syowa station, East Antarctica, and Mario Zucchelli Station, West Antarctica (Fig. 5). Geographically these two sites are separated by 3000 km but the new isolated strains showed 100% similarity in 16S rDNA sequences although different in physiology (Okamoto et al., 2004). For these strains from Antarctica, there existed no closely related type strains, and homology with the most closely related sequences according to the DDBJ/EMBL/GenBank DNA database (including environmental sequences) was as low as 97.7%. This group was first reported by Naganuma et al. (2005), but neither closely related strains nor sequences have been obtained elsewhere as of April 2012, suggesting that it may be an endemic species in the saline ponds of Antarctica.

The genus *Halomonas* is considered cosmopolitan as many species of this genus such as *H. variabilis* are widely distributed (Kaye et al., 2011). However, some strains closely related to

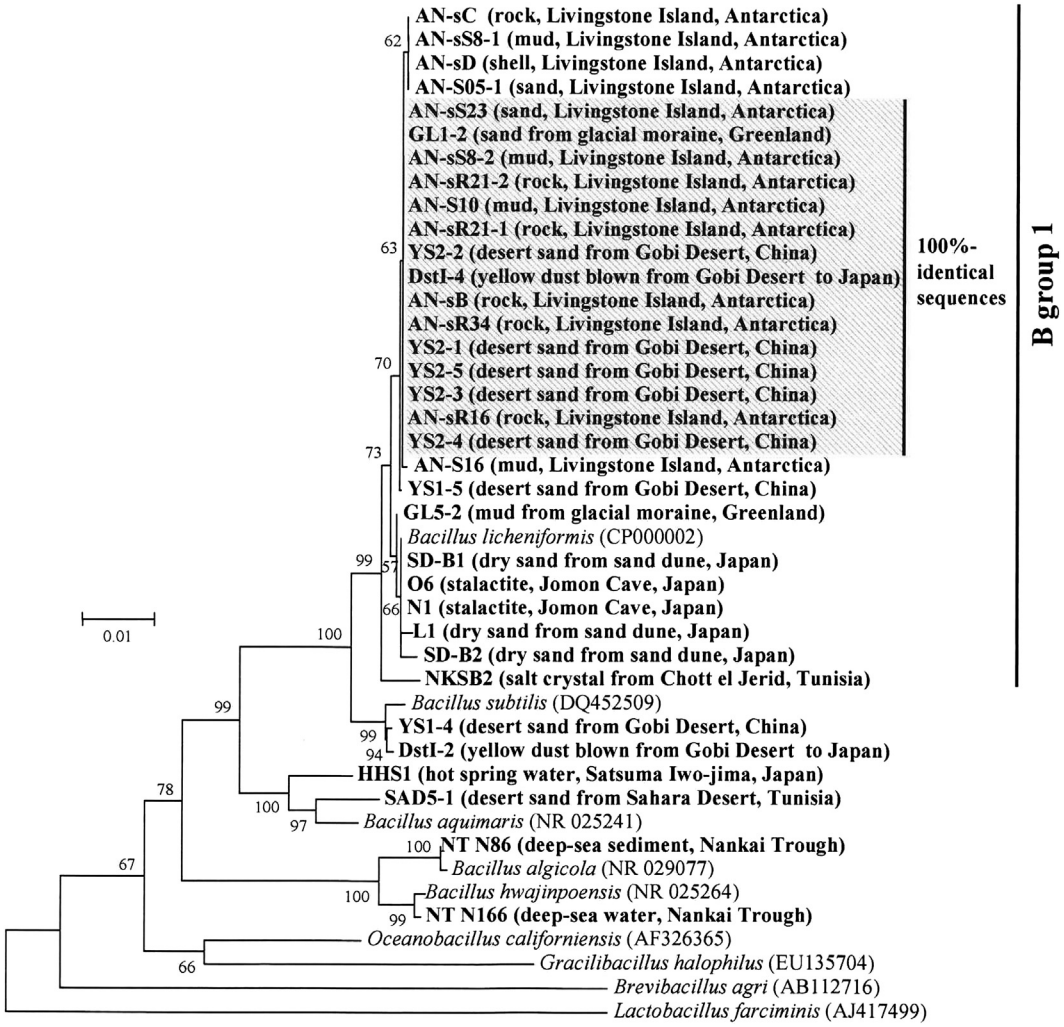


Figure 6. Neighbor-joining phylogenetic tree showing diversity of isolates affiliated to genus *Bacillus* based on near full-length 16S rRNA gene sequences. The percentages of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. There were a total of 1331 positions in the final dataset. Names and origins of isolates are in bold. The scale bar indicates 0.01 substitutions per nucleotide site.

Halomonas were reported only from Antarctica and had highly restricted distribution and could be new species (Naganuma et al., 2005). Similarly a bacterium *Halomonas subglaciescola*, isolated for the first time from Organic Lake in the Vestfold Hills (Franzmann et al., 1987) and never been reported outside of Antarctica could be unique to Antarctica (Vincent, 2000b). These results suggest that *Halomonas* acts as representative of both cosmopolitanism and potential endemism. Some more *Halomonas* spp. reported from Antarctica with neither closely related strains nor sequences reported outside of Antarctica are *Halomonas glaciei* (Reddy et al., 2003) and *Halomonas alkaliantarctica* (Poli et al., 2007) could be endemic to Antarctica. However we cannot consider these novel species to be strictly endemic just because these are unrecorded in other parts of the world. There could be a probability of finding them in other parts of the world. In this case, it is more appropriate to use the term 'potentially endemic' rather than endemic.

3.2. Cosmopolitanism

3.2.1. Isolated strains of spore-forming genus Bacillus

The strains belonging to the genus *Bacillus* are spore-forming. However, unlike some other halophilic genera, not all the strains of *Bacillus* are halophiles. Because of its spore-forming ability, this makes it possible for these microbes to survive under harsh conditions such as extreme temperature, salinity (Yukimura et al., 2009), UV radiations and desiccation (Osman et al., 2008). Nevertheless, one particular species, *B. licheniformis*, is of great interest. Until now it has been isolated from various environments, especially soil, and is tolerant of both salinity and high temperature, and therefore well known as a stress-tolerant bacterium. In this study, we isolated strains that could be classified as *B. licheniformis*, even though at a small number of 28 strains. All isolated strains were closely related to each other and grouped with *B. licheniformis*. However, it is noteworthy that 15 strains were 100% identical in terms of their near full-length 16S rRNA gene sequences (Fig. 6). Strains that were 100% similar were isolated from the following areas: the Gobi Desert in China; yellow dust collected from Japan; the Qaanaaq glacial moraines, Greenland; and Livingston Island in Maritime Antarctica. Beside this study many other studies documented 100% similarity between the 16S rRNA gene sequences of strains collected from different environments and localities. These 100% identical strains isolated over a wide range of habitats across the world suggest the global distribution of *B. licheniformis* strains (Fig. 7).

As evidenced by yellow sand blown into Japan from the Gobi and Taklamakan Deserts, the cross-border and long-distance transport of millions of tons of desert dust annually influences ecosystems on a global scale (Griffin, 2007; Uno et al., 2009). It has also been reported that mineral particles originating from the Taklamakan Desert, one of the sources of yellow dust, are blown all the way to Greenland (Biscaye et al., 1997; Bory et al., 2003). Our research indicates that there may be a chance for long-range transport of halophiles from the mid latitudes to the polar regions. It is easy to conceive that microorganisms are dispersed in the atmosphere in the same manner as dust on account of their small size, and are distributed to various environments as a result. However, not many microorganisms can tolerate the stress of aridity and ultraviolet radiations during atmospheric transport; much less proliferate in their destinations. Evidence suggests that spore-forming halophiles are capable of tolerating such stress, and studies have shown that strains of the family Bacillaceae were even predominant among microorganisms isolated from dust and the atmosphere (Griffin, 2007). This suggests that these halophiles have wider distribution that may have been aided by spore formation ability and successful revival on approach of suitable conditions.

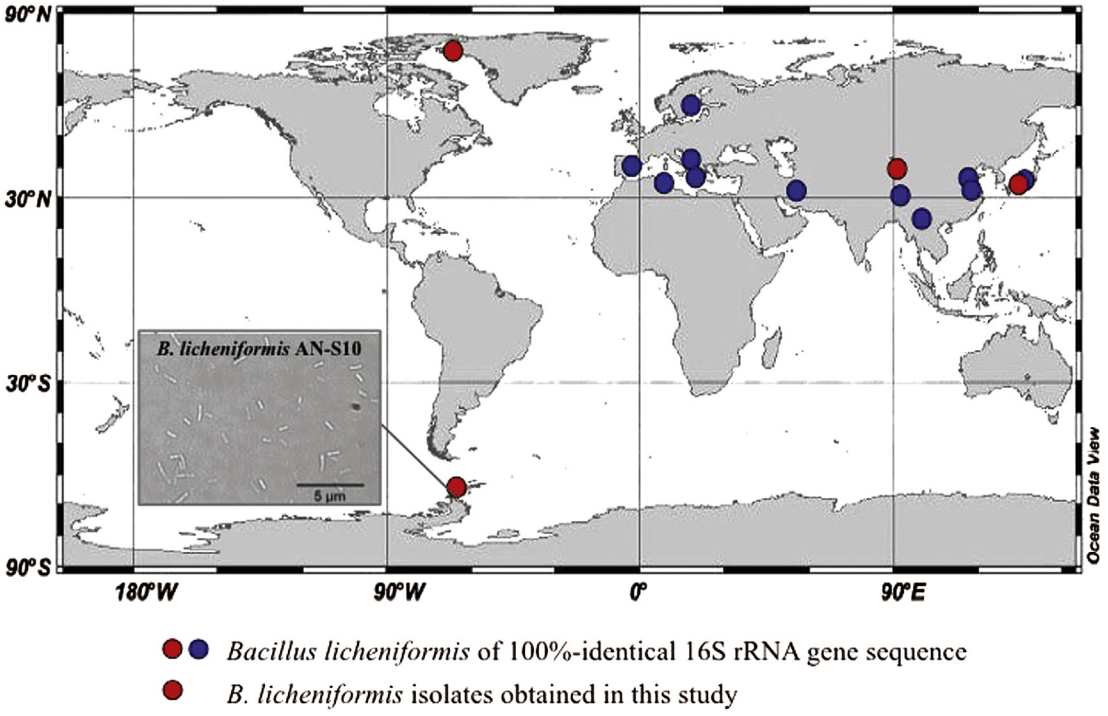


Figure 7. Global distribution of *Bacillus licheniformis* strains. The *Bacillus licheniformis* strains are represented by red circles (isolates obtained in this study) and blue circles (isolates reported in previous studies). These near full-length 16S rRNA gene sequences (1331 bp) were 100% identical to each other. The accession numbers reported in previous studies are as follows: GU568188, FJ514812, AM913927, EU308302, EU073121, AM950314, EF471917, AY479984, AB188216, and DQ228696. This map was generated with Ocean Data View (Schlitzer, 2004) (Black and white) and from google data (colored figure). List of tables with their respective headings.

4. Conclusion

Microbial biogeographic studies in Antarctica are of immense importance to understand their diversity, distribution, evolution and existence of threatened species. The application of many new molecular approaches challenged the ubiquity dictum and provided evidences of microbial biogeography. However, so far the knowledge of microbial biogeography is in its infancy and may sometimes give rise to hazy information due to prevailing limitations of molecular techniques in use. In this study we reviewed the available literature on biogeography of microbes and found that we cannot lump all the microbes under a single biogeographic model. The results of this study suggested potential endemism in some microbes as we isolated one potentially endemic strain from Antarctica. At the same time current investigation suggested that strains closely related to *H. variabilis* have adopted to high salinity and pressure conditions, resulting in their wide distribution range. Similarly the spore-forming ability of *B. licheniformis* helps it to form inactive spores under harsh conditions, which revive on approach of favorable conditions. These microbes, which have developed special survival mechanisms to combat different environmental stress, are found to be cosmopolitan. Hence this study supports the concept of the middle-ground model that suggests some microbes may obey the laws of endemicity while others have cosmopolitan distribution.

Acknowledgments

This work was partly supported by the Special Coordination Fund “Archaean Park Project: International research project on interaction between sub-vent biosphere and geo-environments” of the Ministry of Education, Culture, Sports, Science and Technology of Japan; the NIPR collaborative research program No. 99 (2002–2004); the Fund “Construct the Genetic Resource Library of Unidentified Microbes Based on Genome Information” from the New Energy and Industrial Technology Development Organization, Japan; Grants-in-Aid for Scientific Research (11833012, 14340268, and 18255005) from the Japan Society for the Promotion of Science; and Antarctic samples from Livingston Island were collected during the Spanish program LIMNOPOLAR in the International Polar Year (IPY) 2007–2008 campaign. We are highly appreciative to our colleagues for their help in collecting the samples, including Dr. Synnove Elvevold, Dr. Kim Holmen, Dr. Yoshihide Ohta, Dr. Ana Justel, Dr. Eugenio Rico, Dr. Antonio Quesada and many more.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.gsf.2012.11.002.

References

Amato, P., Hennebelle, R., Magand, O., Sancelme, M., Delort, A.M., Barbante, C., Boutron, C., Ferrari, C., 2007. Bacterial characterization of the snow cover at Spitzberg, Svalbard. Federation of European Microbiological Societies, Microbiology Ecology 59, 55–264.

Ashby, M.N., Rine, J., Mongodin, E.F., Nelson, K.E., Dimster-Denk, 2007. Serial analysis of rRNA genes and the unexpected dominance of rare members of microbial communities. Applied and Environmental Microbiology 73, 4532–4542.

Baas-Becking, L.G.M., 1934. Geobiologie of inleiding tot de milieukunde. In: De Wit, R., Bouvier, T. (Eds.), ‘Everything is Everywhere, But, the Environment Selects’; What did Baas Becking and Beijerinck Really Says?. Environmental Microbiology, vol. 8, pp. 755–758. 4.

Bargagli, R., 2005. Antarctic Ecosystems: Environmental Contamination, Climate Change, and Human Impact. Springer-Verlag, Berlin Heidelberg New York, p. 395.

Biscaye, P.E., Grousset, F.E., Revel, M., Van der Gaast, S., Zielinski, G.A., Vaars, A., Kukla, G., 1997. Asian provenance of glacial dust (stage 2) in the Greenland Ice Sheet Project 2 Ice Core, Summit, Greenland. Journal of Geophysical Research Oceans 102 (C12), 26,765–26,781.

Bory, A.J.M., Biscaye, P.E., Grouseet, F.E., 2003. Two distinct seasonal Asian source regions for mineral dust deposited in Greenland (NorthGRIP). Geophysical Research Letters 30, 1167.

Bowman, J.P., McCammon, S.A., Brown, M.V., Nichols, D.S., McMeekin, T.A., 1997. Diversity and association of psychrophilic bacteria in Antarctic Sea ice. Applied and Environmental Microbiology 63, 3068–3078.

Cánovas, D., Vargas, C., Calderón, M.I., Ventosa, A., Nieto, J.J., 1998. Characterization of the genes for the biosynthesis of the compatible solute ectoine in the moderately halophilic bacterium *Halomonas elongata* DSM3043. Systematic and Applied Microbiology 21 (4), 487–497.

Cho, J., Tiedje, J., 2000. Biogeography and degree of endemicity of fluorescent *Pseudomonas* strains in soil. Applied and Environmental Microbiology 66, 5448–5456.

Christner, B., Skidmore, M., Priscu, J., Tranter, M., Foreman, C., 2008. Bacteria in subglacial environments. In: Margesin, R., Schinner, F. (Eds.), Psychrophiles: From Biodiversity to Biotechnology. Springer-Verlag, Berlin, pp. 51–71.

Clarke, A., 2003. Evolution, adaptation and diversity: global ecology in an Antarctic context. In: Huiskes, A.H.L., Gieskes, W.W.C., Rozema, J., Schorno, R.M.L., van der Vies, S., Wolff, W.J. (Eds.), Antarctic Biology in a Global Context. Backhuys Publishers, Leiden, pp. 3–17.

Cockell, Charles, S., Jones, H.L., 2009. Advancing the case for microbial conservation. Oryx 43 (4), 520–526.

Dahllöf, I., Baillie, H., Kjelleberg, S., 2000. *rpo*B-based microbial community analysis avoids limitations inherent in 16S rRNA gene intraspecies heterogeneity. Applied and Environmental Microbiology 66 (8), 3376–3380.

David, D., Williams, W., 1991. Aerobiology and colonization in Antarctica – the BIOTAS programme. Grana 30 (2), 380–393.

DeLong, E.F., 1992. Archaea in costal marine environments. Proceedings of National Academy of Science USA 89, 5685–5689.

Fenchel, T., Esteban, G.F., Finlay, B.J., 1997. Local versus global diversity of microorganisms: cryptic diversity of ciliated protozoa. Oikos 80, 220–225.

Fenchel, T., Finlay, B.J., 2004. The ubiquity of small species: patterns of local and global diversity. BioScience 54, 777–784.

Fendrich, C., 1988. *Halovibrio variabilis* gen. nov. sp. nov., *Pseudomonas halophila* sp. nov. and new halophilic aerobic coccoid Eubacterium from Great Salt Lake, Utah, USA. Systematic and Applied Microbiology 11, 36–43.

Fernandez, R., Hodgson, D.A., Convey, P., Wilmotte, A., 2011. Low cyanobacterial diversity in biotopes of the transantarctic mountains and shackleton range (80–82°S), Antarctica. Federation of European Microbiological Societies, Microbiology Ecology 77, 503–517.

Finlay, B.J., 2001. Protozoa. Encyclopedia of Biodiversity 4, 901–915.

Foissner, W., 2006. Biogeography and dispersal of micro-organisms: a review emphasizing protists. Acta Protozoologica 45, 111–136.

Foissner, W., 2007. Dispersal and biogeography of protists: recent advances. The Japanese Journal of Protozoology 40 (1), 1–16.

Foissner, W., Agatha, S., Berger, H., 2002. Soil ciliates (Protozoa, Ciliophora) from Namibia (Southwest Africa), with emphasis on two contrasting environments, the Etosha region and the Namib Desert. Denisia 5, 1–1459.

Franzmann, P.D., Burton, H.R., McMeekin, T.A., 1987. Halomonas subglaciescola, a new species of halotolerant bacteria isolated from Antarctica. International Journal of Systematic and Evolutionary Bacteriology 37, 27–34.

Galinski, E.A., 1995. Osmoadaptation in Bacteria. Advances in Microbial Physiology 37, 273–328.

Gaston, K.J., 1998. Rarity as double jeopardy. Nature 394, 229–230.

Gibson, J.A.E., Wilmotte, A., Taton, A., Van De Vijer, B., Beyens, L., Dartnall, H.J.G., 2006. Biogeographic trends in Antarctic lake communities. In: Bergstrom, D.M., Convey, P., Huiskes, A.H.L. (Eds.), Trends in Antarctic Terrestrial and Limnetic Ecosystems. Springer, pp. 71–99.

Griffin, D.W., 2007. Atmospheric movement of microorganisms in clouds of desert dust and implications for human health. Clinical Microbiology Reviews 20 (3), 459–477.

Hobbie, J.E., Laybourn-Parry, J., 2008. Heterotrophic microbial processes in polar lakes. In: Vincent, W.F., Laybourn-Parry, J. (Eds.), Polar Lakes and Rivers: Limnology of Arctic and Antarctic Aquatic Ecosystem. Oxford University Press Inc., New York, pp. 197–212.

Hua, N.P., Kobayashi, F., Iwasaka, Y., Shi, G.Y., Naganuma, T., 2007. Detailed identification of desert-originated bacteria carried by Asian dust storms to Japan. Aerobiologia 23, 291–298.

James, S.R., Burton, H.R., McMeekin, T.A., Mancuso, C.A., 1994. Seasonal abundance of *Halomonas meridiana*, *Halomonas subglaciescola*, *Flavobacterium gondwanense*, and *Flavobacterium salegens*. Antarctic Science 6, 325–332.

Jenkins, D.G., Brescacin, C.R., Duxbury, C.V., Elliott, J.A., Evans, J.A., Grablow, K.R., Hillegass, M., Lyon, B.N., Metzger, G.A., Olandese, M.L., Pepe, D., Silvers, G.A., Suresch, H.N., Thompson, T.N., Trexler, C.M., Williams, G.E., Williams, N.C., Williams, S.E., 2007. Does size matter for dispersal distance? Global Ecology and Biogeography 16, 415–425.

Jones, A., Harrison, R., 2004. The effects of meteorological factors on atmospheric bioaerosol concentrations - a review. Science of Total Environment 326, 151–180.

Jungblut, A.D., Hawes, I., Mountfort, D., Hitzfeld, B., Dietrich, D.R., Burns, B.P., Neilan, B.A., 2005. Diversity within cyanobacterial mat communities in variable salinity meltwater ponds of McMurdo ice shelf, Antarctica. Environmental Microbiology 7 (4), 519–529.

Jungblut, A.D., Lovejoy, C., Vincent, W.F., 2010. Global distribution of cyanobacterial ecotypes in the cold biosphere. The ISME Journal 4, 191–202.
Kaye, J.Z., Baross, J.A., 2000. High incidence of halotolerant bacteria in Pacific hydrothermal-vent and pelagic environments. FEMS Microbiology Ecology 32, 249–260.
Kaye, J.Z., Sylvan, A.B., Edwards, K.J., Baross, J.A., 2011. Halomonas and Marinobacter ecotypes from hydrothermal vent, subseafloor and deep-sea environments. Federation of European Microbiological Societies, Microbiology Ecology 75 (1), 123–133.
Komárek, J., 1999. Diversity of cyanoprokaryotes (cyanobacteria) of King George Island, maritime Antarctica – a survey. Archiv für Hydrobiologie 94, 181–193.
Komárek, J., Elster, J., Komárek, O., 2008. Diversity of the cyanobacterial microflora of the northern part of James Ross Island, NW Weddell Sea, Antarctica. Polar Biology 31, 853–865.
Krieg, N.R., 1981. Enrichment and isolation. In: Gerhardt, P., Murray, R.G.E., Costilow, R.N., Nester, W.W., Wood, W.A., Krieg, N.R., Phillips, G.B. (Eds.), Manual of Methods for General Bacteriology. American Society for Microbiology, Washington, DC, pp. 328–364.
Larkin, M.A., Blackshields, G., Brown, N.P., Chenna, R., McGettigan, P.A., McWilliam, H., Valentin, F., Wallace, I.M., Wilm, A., Lopez, R., Thompson, J.D., Gibson, T.J., Higgins, D.G., 2007. ClustalW and ClustalX version 2.0. Bioinformatics 23, 2947–2948.
Laybourn-Parry, J., 2002. Survival mechanisms in Antarctic lakes. Philosophical Transactions of the Royal Society Biological Sciences 357, 863–869.
Laybourn-Parry, J., Pearce, D.A., 2007. The biodiversity and ecology of Antarctic lakes: models for evolution. Philosophical Transactions of the Royal Society 362, 2273–2289.
Litchman, E., 2010. Invisible invaders: non-pathogenic invasive microbes in aquatic and terrestrial ecosystems. Ecology Letters 13, 1560–1572.
Margesin, R., Miteva, V., 2011. Diversity and ecology of psychrophilic microorganisms. Research in Microbiology 162, 346–361.
Marshall, W., Laybourn-Parry, J., 2002. The balance between photosynthesis and grazing in Antarctic mixotrophic cryptophytes. Freshwater Biology 47, 2060–2070.
Martiny, J.B.H., Bohannan, B.J.M., Brown, J.H., Colwell, R.K., Fuhrman, J.A., Green, J.L., Horner-Devine, M.C., Kane, M., Krumins, J.A., Kuske, C.R., Morin, P.J., Naeem, S., Øvreås, L., Reysenbach, A.L., Smith, V.H., Staley, J.T., 2006. Microbial biogeography: putting microorganisms on the map. Nature Reviews Microbiology 4, 102–112.
Mergaert, J., Verhelst, A., Cnockaert, M.C., Tan, T.L., Swings, J., 2001. Characterization of facultative oligotrophic bacteria from polar seas by analysis of their fatty acids and 16S rDNA sequences. Systematic and Applied Microbiology 24, 98–107.
Moosvi, S.A., McDonald, I.R., Pearce, D.A., Kelly, D.P., Wood, A.P., 2005. Molecular detection and isolation from Antarctica of methylotrophic bacteria able to grow with methylated sulfur compounds. Systematic and Applied Microbiology 28 (6), 541–554.
Myers, N., Mittermeier, R.A., Mittermeier, C.G., da Fonseca, G.A.B., Kent, J., 2000. Biodiversity hotspots for conservation priorities. Nature 403, 853–858.
Nadeau, T.L., Milbrandt, E.C., Castenholz, R.W., 2001. Evolutionary relationships of cultivated Antarctic Oscillatorians (Cyanobacteria). Journal of Phycology 37, 650–654.
Naganuma, T., Hua, P.N., Okamoto, T., Ban, S., Imura, S., Kanda, H., 2005. Depth distribution of euryhaline halophilic bacteria in Suribati Ike, a meromictic lake in East Antarctica. Polar Biology 28, 964–970.
Naganuma, T., Wilmotte, A., 2009. Microbiological and ecological responses to global environmental changes in polar regions (MERGE): an IPY core coordinating project. Polar Science 3, 139–146.
Nakai, R., Shibuya, E., Justel, A., Rico, E., Quesada, A., Kobayashi, F., Iwasaka, Y., Shi, G.Y., Amano, Y., Iwatsuki, T., Naganuma T. Phylogeographic analysis of 0.2-μm-filterable bacteria with special reference to Rhizobiales strains that occur at cryospheric habitats. *Antarctic Science*, in press.
Namsaraev, Z., Mano, M.J., Fernandez, R., Wilmotte, A., 2010. Biogeography of terrestrial cyanobacteria from Antarctic ice-free areas. Annals of Glaciology 51 (56), 171–177.
Nemergut, D.R., Costello, E.K., Hamady, M., Lozupone, C., Jiang, L., Schmidt, S.K., Fierer, N., Townsend, A.R., Cleveland, C.C., Stanish, L., Knight, R., 2011. Global patterns in the biogeography of bacterial taxa. Environmental Microbiology 13 (1), 135–144.
Okamoto, T., Fujioka, K., Naganuma, T., 2001. Phylogenetic similarity of aerobic gram-negative halophilic bacteria from deep-sea hydrothermal mound and Antarctic habitats. Polar Bioscience 14, 1–9.
Okamoto, T., Maruyama, A., Imura, S., Taleyama, H., Naganuma, T., 2004. Comparative phylogenetic analyses of *Halomonas variabilis* and related organisms based on 16S rRNA, *gyrB* and *etcBC* gene sequence. Systematic and Applied Microbiology 27, 323–333.
Onyenwoke, R.U., Brill, J.A., Wiegel, K.F.J., 2004. Sporulation genes in members of the low G + C Gram-type-positive phylogenetic branch (*Firmicutes*). Archives of Microbiology 182, 182–192.
Orphan, V.J., Taylor, L.T., Hafenbradl, D., DeLong, E.F., 2000. Culture-dependent and culture-independent characterization of microbial assemblages associated with high-temperature petroleum reservoirs. Applied and Environmental Microbiology 66, 700–711.
Osman, S., Peeters, Z., La Duc, M.T., Mancinelli, R., Ehrenfreund, P., Venkateswaran, K., 2008. Effect of shadowing on survival of bacteria under conditions simulating the Martian atmosphere and UV radiation. Applied and Environmental Microbiology 74 (4), 959–970.
Papke, R.T., Ward, D.M., 2004. The importance of physical isolation to microbial diversification. FEMS Microbiology Ecology 48, 293–303.
Petz, W., Valbonesi, A., Schiftner, U., Quesada, A., Ellis-Evans, C., 2007. Ciliate biogeography in Antarctic and Arctic freshwater ecosystems: endemism of global distribution of species? Federation of European Microbiological Societies 59, 396–408.
Poli, A., Esposito, E., Orlando, P., Lama, L., Giordano, A., de Appolonia, F., Nicolaus, B., Gambacorta, A., 2007. Halomonas alkaliantarctica sp. nov., isolated from saline lake Cape Russell in Antarctica, an alkalophilic moderately halophilic, exopolysaccharide-producing bacterium. Systematic and Applied Microbiology 30 (1), 31–38.
Pross, J., Contreras, L., Bijl, P.K., Greenwood, D.R., Bohaty, S.M., Schouten, S., Bendle, J.A., Rohl, U., Tauxe, L., Raine, J.I., Huck, C.E., van de Flierdt, T., Jamieson, S.S.R., Stickley, C.E., van de Schootbrugge, B., Escutia, C., Brinkhuis, H., 2012. Persistent near-tropical warmth on the Antarctic continent during the early Eocene epoch. Nature 488, 73–77.
Ragon, M., Fontaine, M.C., Moreira, D., Lopezgarcia, P.N., 2012. Different biogeographic patterns of prokaryotes and microbial eukaryotes in epilithic biofilms. Molecular Ecology 21, 3852–3868.
Reddy, G.S., Raghavan, P.U., Sarita, N.B., Prakash, J.S., Nagesh, N., Delille, D., Shivaji, S., 2003. Halomonas glaciei sp. nov. isolated from fast ice of Adelie Land, Antarctica. Extremophiles 7 (1), 55–61.
Rossello-Mora, R., Lucio, M., Pena, A., Brito-Echeverría, J., López-López, A., Valens-Vadell, M., Frommberger, M., Antón, J., Schmitt-Kopplin, P., 2008. Metabolic evidence for biogeographic isolation of the extremophilic bacterium *Salinibacter ruber*. ISME Journal 2, 242–253.
Saitou, N., Nei, M., 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Molecular Biology and Evolution 4 (4), 406–425.
Sass, A.M., Sass, H., Coolen, M.J.L., Cypionka, H., Overmann, J., 2001. Microbial communities in the chemocline of a hypersaline deep-sea basin (Urania basin, Mediterranean Sea). Applied and Environmental Microbiology 67 (12), 5392–5402.
Schlitzer, R., 2004. Ocean Data View, 3.43 ed. Alfred Wegener Institute for Polar and Marine Research, Bremerhaven, Germany.
Severin, I., Stal, L.J., 2010. Diazotrophic microbial mats. In: Seckbach, J., Oren, A. (Eds.), Microbial Mats: Modern and Ancient Microorganisms in Stratified Systems, Cellular Origin, Life in Extreme Habitats and Astrobiology, vol. 14. Springer, pp. 321–339.
Shah, N., Tang, H., Doak, T.G., Ye, Y.Z., 2011. Comparing bacterial communities inferred from 16S rRNA gene sequencing and shotgun metagenomics. Pacific Symposium on Biocomputing 16, 165–176.
Shivaji, S., Reddy, G.S.N., Aduri, R.P., Kutty, R., Ravenschlag, K., 2004. Bacterial diversity of soil sample from Schirmacher oasis, Antarctica. Cellular and Molecular Biology 50 (5), 525–536.
Staley, J.T., 1997. Biodiversity: are microbial species threatened? Current Opinion in Biotechnology 8, 340–345.
Staley, J.T., Gosinks, J.J., 1999. Poles apart: biodiversity and biogeography of sea ice bacteria. Annual Review of Microbiology 53, 189–215.
Tamura, K., Dudley, J., Nei, M., Kumar, S., 2007. MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. Molecular Biology and Evolution 24, 1596–1599.
Taton, A., Grubisic, S., Brambilla, E., De-Wit, R., Wilmotte, A., 2003. Cyanobacterial diversity in natural and artificial microbial mats of Lake Fryxell (McMurdo Dry Valleys, Antarctica): a morphological and molecular approach. Applied and Environmental Microbiology 69 (9), 5157–5169.
Taton, A., Grubisic, S., Balthasart, P., Hodgson, D.A., Laybourn-Parry, J., Wilmotte, A., 2006. Biogeographical distribution and ecological ranges of benthic cyanobacteria in East Antarctic lakes. Federation of European Microbiological Societies, Microbiology Ecology 57, 272–289.
Uno, I., Eguchi, K., Yumimoto, K., Takemura, T., Shimizu, A., Uematsu, M., Liu, Z., Wang, Z., Hara, Y., Sugimoto, N., 2009. Asian dust transported one full circuit around the globe. Nature Geoscience 2, 557–560.
Van Trappen, S., Mergaert, J., Van Eygen, S., Dawyndt, P., Cnockaert, M.C., Swings, J., 2002. Diversity of 746 heterotrophic bacteria isolated from microbial mats from ten Antarctic lakes. Systematic and Applied Microbiology 25, 603–610.
Vanormelingen, P., Verleyen, E., Vyverman, W., 2008. The diversity and distribution of diatoms: from cosmopolitanism to narrow endemism. Biodiversity and Conservation 17, 393–405.
Vincent, W.F., 2000a. Cyanobacterial dominance in the polar regions. In: Whitton, B.A., Potts, M. (Eds.), The Ecology of Cyanobacteria. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 321–340.
Vincent, W.F., 2000b. Evolutionary origins of Antarctic microbiota: invasion, selection and endemism. Antarctic Science 12 (3), 374–385.
Vyverman, W., Verleyen, E., Sabbe, K., Vanhoutte, K., Sterken, M., Hodgson, D., Mann, D.G., Juggins, S., Van de Vijver, B., Jones, V.J., Flower, R., Roberts, D.A., Chepurnov, V.A., Kilroy, C., Vanormelingen, P., 2007. Historical processes constrain patterns in global diatom diversity. Ecology 88, 1924–1931.
Vyverman, W., Verleyen, E., Wilmotte, A., Hodgson, D.A., Willems, A., Peeters, K., deVijver, B.,V., Wever, A.D., Leliaert, F., Sabbe, K., 2010. Evidence for widespread endemism among Antarctic micro-organisms. Polar Science 4, 103–113.

Watts, P.C., Martin, L.E., Kimmance, S.A., Montagnes, D.J.S., Lowe, C.D., 2011. The distribution of *Oxyrrhis marina*: a global disperser or poorly characterized endemic? Journal of Plankton Research 33 (4), 579–589.

Whitaker, R.J., 2006. Allopatric origins of microbial species. Philosophical Transactions of the Royal Society B-Biological Sciences 361, 1975–1984.

Whitaker, R.J., Grogan, D.W., Taylor, J.W., 2003. Geographic barriers isolate endemic populations of hyperthermophilic archaea. Science 301, 976–978.

Whitfield, J., 2005. Biogeography: is everything everywhere? Science 310, 960–961.

Whitman, W.B., Coleman, D.C., Wiebe, W.J., 1998. Prokaryotes: the unseen majority. Proceeding of National Academy of Science, USA 95, 6578–6583.

Wilkinson, D.M., Koumoutsaris, S., Mitchell, E.A.D., Bey, I., 2012. Modelling the effect of size on the aerial dispersal of microorganisms. Journal of Biogeography 39 (1), 89–97.

Yukimura, K., Nakai, R., Kohshima, S., Uetake, J., Kanda, H., Naganuma, T., 2009. Spore-forming halophilic bacteria isolated from Arctic terrains: implications for long-range transportation of microorganisms. Polar Science 3, 163–169.

Zakhia, F., Jungblut, A.D., Taton, A., Vincent, W.F., Wilmotte, A., 2008. Cyanobacteria in cold ecosystems. In: Margesin, R., Schinner, F., Marx, J.C., Gerday, C. (Eds.), Psychrophiles: From Biodiversity to Biotechnology. Springer-Verlag, Berlin Heidelberg, pp. 121–135.